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(54) Title: FRACTIONATION OF HEMICELLULOSIC MATERIALS

(57) Abstract

Disclosed is a process that uses selective enzymes from microbial or plant sources to facilitate the extraction of hemicellulose. Selective removal of acetate using acetyl xylan esterase (E.C.3.1.1.6) under conditions that will not hydrolyse the feruoylate esters. This treatment renders the arabinoxylan ferulate soluble under mild aqueous conditions without the use of harsh alkalis. Arabinoxylan ferulate is extracted intact and subsequently the level of ferulic acid can be controlled to give the degree of crosslinking required by treatment of the soluble arabinoxylan ferulate with ferulic acid esterase.

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TITLE

FRACTIONATION OF HEMICELLULOSIC MATERIALS

FIELD OF THE INVENTION

The present invention relates to processes for extracting and modifying various compositions obtainable from plant tissue, and in particular to processes involving the enzymic modification of hemicelluloses. In particular, the invention relates to processes (and particularly industrial processes) for the production of a variety of products and co-products generated from the enzymic treatment and subsequent processing of various hemicellulosic starting materials (including gelling and non-gelling hemicelluloses, gelled hemicelluloses, cellulosic fibre, proteins and phenolic extracts) which have a wide variety of uses in the food and medical industries and in agriculture.

BACKGROUND OF THE INVENTION

Plant tissue may be classified into eight major components, listed below:

1. Cellulose
2. Hemicellulose
3. β-glucan
4. Starch
5. Protein
6. Phenolic acids
7. Lignin
8. Waxes, cutin and suberin.

Each of these components are briefly discussed in turn below:

Cellulose

Cellulose is a polymer of (1-4)-linked β-glucopyranosyl residues containing thousands of glucosyl residues per chain. Individual cellulose chains form a twofold helix (two glucosyl residues per turn of the helix), and the two hydrogen bonds between adjacent glucosyl residues "lock" the polysaccharide chain into an extended, ribbonlike, and relatively inflexible conformation.

The extended cellulose chains align and aggregate to form crystalline microfibrils of up to 25 nm in diameter; in native cellulose, the cellulose chains in the microfibrils are believed to be organized in a parallel orientation with extensive intermolecular hydrogen bonding. Within a microfibril, regions of high and relatively low crystallinity coexist.

Cellulose is usually obtained as an insoluble fibrous residue following the alkaline and/or water extraction of vegetable material.

Hemicellulose

The term "hemicellulose" is a term of art used to embrace non-cellulosic, non-starch plant polysaccharides. The term therefore embraces *inter alia* pentosans, pectins and gums.

Some hemicelluloses are suitable as substrates for oxidative gelation ("gelling hemicelluloses"): such hemicelluloses often have substituents with phenolic groups which are cross-linkable with certain oxidizing agents.

Arabinoxylan and pectin constitute two particularly important classes of hemicellulose. Arabinoxylans consist predominantly of the pentoses arabinose and xylose, and are therefore often classified as pentosans. However, in many cases hexoses and hexuronic acid are present as minor constituents, and therefore they may also be referred to descriptively as heteroxylans.

The arabinoxylan molecule consists of a linear backbone of (1-4)-β-xylopyranosyl units, to which substituents are attached through O2 and O3 atoms of the xylosyl residues. The major substituents are single α-L-arabinofuranosyl residues. Single α-D-glucoronopyranosyl residues and their 4-*O*-methyl ethers are also common substituents.

Arabinoxylan preparations are usually heterogeneous with respect to the ratio of xylose to arabinose (i.e., the degree of substitution) and in the pattern of substitution of the arabinosyl units along the (1-4)-β-xylan backbone.

Phenolic acid (including ferulic acid) and acetyl substituents occur at intervals along the arabinoxylan chains. These substituents to some extent determine the solubility of the arabinoxylan. Arabinoxylan preparations bearing phenolic (e.g., ferulic acid substituents) are referred to herein as "AXF", while those bearing acetyl substituents are designated "AXA". Similarly, preparation bearing both phenolic (e.g., ferulic acid) and acetyl substituents are hereinafter abbreviated to the designation "AXFA". Arabinoxylan preparations having few phenolic (e.g., ferulic acid) substituents are designated "AX": when the degree of substitution falls below that required for oxidative gelation, the arabinoxylan is designated a "non-gelling arabinoxylan" (a term which therefore embraces AX and AXA).

Pectins constitute another important class of hemicelluloses. As used herein and unless otherwise indicated, the term "pectin" is used *sensu lato* to define hemicellulose polymers rich in D-galacturonic acid. Many (but not all) are cell wall components. The term "pectin" is also used herein *sensu stricto* to define the so-called "true pectins", which are characterized by the presence of an *O*-(α-D-galacturonopyranosyl)-(1-2)-L-rhamnopyranosyl linkage within the molecule.

The pectins may be subcategorized on the basis of their structural complexity. At one extreme are "simple pectins", which are galacturonans. At the other extreme are "complex pectins" exemplified by rhamnogalacturonan II, which contains at least 10 different monosaccharide components in the main chain or as a components of branches. Pectins of intermediate complexity (herein referred to as "mesocomplex pectins" contain alternate rhamnose and galacturonic acid units, while others have branches of glucoronic acid linked to galacturonic acid.

Complex and mesocomplex pectins are made up of "smooth" regions (based on linear homogalacturonan) and "hairy" regions corresponding to the rhamnogalacturonan backbone with side-branches of varying length.

Certain pectins (for example, pectins obtainable from representatives of the plant family *Chenopodiaceae*, which include beets (e.g., sugar beet), spinach and mangelwurzels) are substituted to some extent with substituents derived from carboxylic acids (usually substituted cinnamic acids) containing phenolic groups. Such pectins may be oxidatively cross-linked to produce viscous solutions or gels *via* their phenolic substituents. This can be achieved by powerful oxidants (e.g., persulfate - see J.-F. Thibault *et alia*, in The Chemistry and Technology of Pectin, Academic Press 1991, Chapter 7, pages 119-133) or a combination of peroxidase and hydrogen peroxide (see Thibault *et alia*, *ibidem*). FR 2 545 101 A1 also describes the gelling of beet pectins using an oxidant (e.g., hydrogen peroxide) and an enzyme (peroxidase). Such pectins are referred to herein as "gelling pectins".

Sugar beet pectin is especially rich in arabinan. Arabinan contains β-1,5-linked arabinose in the backbone with a-(1->3) or a-(1->2)-linked arabinose residues, whereas arabinogalactan contains β-1,4-linked galactose in the backbone, with a-(1->3) or a-(1->2) linked arabinose residues. Ferulyl substituents are linked to the arabinose and/or the galactose in the arabinan and arabinogalactan side-branches of the rhamnogalacturonan part. The "ferulic acid" content varies according to the extraction method, but is often about 0.6%.

Beet pectins obtained by processes which partially remove arabinose residues may exhibit improved gelling properties. Thus, procedures involving mild acid treatment and/or treatment with an a-arabinofuranosidase will improve the gelling properties of the pectin (see F. Guillon and J.-F. Thibault, *ibidem*). Such pectins are hereinafter referred to as "treated pectins".

β-Glucan

The (1-3, 1-4)-β-glucans consist of linear chains of β-glucosyl residues joined by both (1-3) and (1-4)-glycosidic linkages. Minor amounts of arabinosyl and/or xylosyl residues may be covalently linked to these chains. The β-glucans appear to occur as a family of (1-3, 1-4)-β-glucans with different molecular sizes and fine structures within the plant.

Starch

Starch is the major storage product of the world's most important food crops and is found in large quantities in the seeds of cereals (such as wheat, corn and rice), in legumes (such as pea) and in tuber and root crops such as potato and yam. It is laid down in all higher plants in the form of insoluble grains or granules that act as an energy reserve. The starch granule usually comprises two different polymers: amylose (an essentially linear chain of a(1-4)-linked α-*D*-glucopyranosyl residues) and amylopectin (which comprises highly

branched a(1-4)-linked α-*D*-glucopyranosyl residues, the branching occurring *via* a(1-6) linkages). Together, amylose and amylopectin make up 97-99% of the dry weight of starch. Other minor constituents include lipids (principally occurring in cereal starches), protein and trace elements (e.g., phosphorous).

Two crystalline forms of amylose can be identified: the so-called "A" and "B" forms. The "B" form is formed during retrogradation at room temperature, while the "A" form can be grown under other conditions (for example, at temperatures above 50°C). The "A" form can also be produced with short chain amylose, which as used herein defines amylose having a molecular weight distribution sufficiently small such that "A" type crystals preferentially form. Both "A" and "B" forms are believed to form crystals based on regular parallel packing of amylose double helices, with the different forms having different unit cells in which the packing leads to significant differences in the positioning of water (in the "A" crystals, four water molecules are located between the helices, as oppose to thirty six in the "B" form).

Amylose has very limited branching (about one branch point for every few thousand glucose units). Molecular weights are typically around 10^5-10^6. Amylopectin is much more branched than amylose, with typically 5% of the glucose units containing α(1-6)-linked branch points to connect the α(1-4)-linked chains. The molecular weight is much higher than amylose, typically in excess of 10^8.

The amylose contents of different starches varies. Potato and tapioca starch typically have much lower amylose contents (21% and 17%, respectively) than the 28% found in maize and wheat starch. Amylose can complex with lipid, wherein the lipid molecule reside within a single helix of amylose. Such complexes are termed helical inclusion complexes.

Protein

These include storage proteins, enzymes, structural proteins (e.g., associated with testaceous plant material), glycoproteins and arabinogalactan-proteins. These latter proteins comprise a hydroxyproline-rich polypeptide backbone covalently attached through β-galactosyl-hydroxyproline linkages to a branched β-galactan that bears arabinofuranosyl substituents.

Plant protein may also comprise protein covalently associated with polysaccharide chains, for example to starch, β-glucan or arabinoxylan.

Enzymes may include peroxidases, oxidases, invertases, malate dehydrogenases, ferulic acid esterases and acetyl esterases (e.g., acetyl xylan esterases).

Phenolic acids

The phenolic acids (chiefly ferulic and *p*-coumaric acids) are common in cell walls from cereal grains and have also been detected in barley husks and embryo. They may be attached to barley storage proteins and are found in starchy endosperm cell walls and in the aleurone layer. The phenolic acids (e.g., ferulic acid) may be associated with

polysaccharides (such as hemicelluloses, e.g., arabinoxylans), where they may be cross-linkable by oxidative gelation (see *infra*). The phenolic aldehydes *p*-hydroxybenzaldehyde, vanillin and syringaldehyde have been identified in cell walls of grasses and are apparently linked at their phenolic groups.

Lignin

Lignin is a copolymer of three phenylpropanoid molecules: coniferyl, sinapyl and *p*-hydroxycinnamyl alcohols, the proportion of which varies between different plant species. The monomeric alcohols are joined by several types of covalent linkages in the lignin polymer, while lignin itself is believed to be linked covalently to cell wall matrix polysaccharides.

Waxes, cutin and suberin

Many plant structures (e.g., the outermost envelope of the husk and the pericarp in cereals such as wheat, rice and barley) are covered by a cuticle attached to the epidermal cell walls. The cuticle comprises the polyester cutin, embedded in a mixture of nonpolar lipids (waxes). The cuticle presents a barrier to the diffusion of water, solutes and other molecules; the waxes provide the major diffusion barrier.

A second permeability barrier is found in seed coats, the suberized layer. Suberin consists of aliphatic fatty acids and aromatic monomers such as *p*-coumaric and ferulic acid.

Oxidative gelation, gelling hemicelluloses and hemicellulose gels

Aqueous extracts of several different types of hemicelluloses are known to form gels (or viscous liquids) when treated with certain oxidizing agents. For example, it has long been known that certain flour extracts (e.g., wheat and rye flour extracts) can form gels in the presence of certain oxidants (e.g., upon the addition of hydrogen peroxide).

The phenomenon is known in the art as "oxidative gelation", and an extensive literature exists on the subject of oxidative gelation of wheat flour extracts. The term "oxidative gelation" is used herein in a broad sense to include the case where viscous solutions are produced rather than true gels, and the term "gel" is therefore to be interpreted loosely to cover viscous liquids. This reflects the fact that oxidative gelation is a progressive phenomenon which may be controlled to vary the degree of gelation to the extent that hard, brittle gels are formed at one extreme and slurries or viscous liquids at the other.

The biochemical basis of the gelling process is not completely or consistently described in the prior art. According to one model, the gels arise as high molecular weight arabinoxylan and protein molecules become inter- and/or intra-linked (*via inter alia* phenolic substituents, for example ferulic acid-derived diferulate bridges): see e.g., Hoseney and Faubion (1981), Cereal Chem., 58:421.

In another model, gel formation and/or viscosity increases arise (at least in part) from cross-linking within and/or between macromolecular components of the hemicellulose

mediated by ferulic acid residues (for example, involving diferulate generated by oxidative coupling of the aromatic nucleus of ferulic acid).

It should be noted that, as used herein (and as is usual in the art), the terms "ferulic acid" and "ferulate" are used *sensu lato* encompass feruloyl (often denoted feruloyl) groups (i.e., 4-hydroxy-3-methoxy-cinnamyl groups) and derivatives (particularly oxidized derivatives) thereof.

Only a few oxidizing agents are known to have the ability to induce gelation, and these include hydrogen peroxide (usually in conjunction with a peroxidase), ammonium persulphate and formamidine disulphide.

Most of the work in the area of oxidative gelation has focused on water soluble pentosans from wheat flour. In these studies, wheat flour is extracted with water (usually at room temperature) to yield gelling arabinoxylans. However, water-insoluble wheat pentosans extracted from wheat flours with various concentrations of cold sodium hydroxide have also been shown to form gels (Michniewicz *et alia*, Cereal Chemistry 67(5): 434-439 (1990), and oxidative gelation of beet pectins has also been described: see J.-F. Thibault *et alia*, in The Chemistry and Technology of Pectin, Academic Press 1991, Chapter 7, pages 119-133) and FR 2 545 101 A1, discussed earlier.

WO 93/10158 describes the preparation of hemicellulosic material from various brans and the oxidative gelation of maize-derived hemicelluloses using an oxidizing system comprising a peroxide (such as hydrogen peroxide) and an oxygenase (such as a peroxidase). The hemicellulosic material for use as a gelling agent is prepared by hot water or mild alkali extraction.

WO 96/03440 describes the use of an oxidase (preferably a laccase) for promoting oxidative gelation of *inter alia* arabinoxylans. However, laccase may not be acceptable for use in certain food applications, is relatively expensive and the supply is limited. Moreover, oxidases such as laccase are relatively weak oxidation-promoters, and the range of different gel strengths obtainable by the use of such enzymes is limited. Indeed, it is possible that the crosslinking achieved through the use of laccase and other oxidases differs fundamentally from that mediated by e.g., hydrogen peroxide, so that the gels may differ significantly in structure from those produced by other forms of oxidative gelation.

SUMMARY OF THE INVENTION

There are many known methods for fractionating plant material (such as testaceous or cell wall material) to produce gelling hemicelluloses. Such methods usually involve alkali and/or water extraction to yield insoluble cellulose and soluble hemicellulose fractions, followed by separation. The soluble extract is then often neutralized (or acidified) to precipitate hemicelluloses.

Organic solvents are also commonly used instead of (or in addition to) acidification to precipitate further hemicellulose fractions.

In the past, gelling hemicelluloses such as arabinoxylan ferulate have been isolated from plants or hemicellulosic starting material by extracting into water or alkaline solutions. Extensive hydrolysis (by e.g., harsh alkaline treatments) is known to strip the ferulic acid residues from the bulk pentosans, and so hemicelluloses for use as starting materials in the production of gels or viscous solutions are usually extracted by water (particularly hot water) or mild alkali extraction.

However, water extraction can be used only with a relatively small class of gelling hemicelluloses (and so such extractions are not generally applicable), while even mild alkaline extraction procedures physically alter cell wall polymers (for example, by disrupting hydrogen bonds and inducing ionization and consequent conformational changes). Alkaline media are also degradative, and lead to disruption of covalent bridging structures holding polymers in the cell wall and to chemical changes in the polymers themselves. Thus, cleavage of ester linkages and peptide bonds and polysaccharide depolymerization by alkaline peeling (β-elimination) at the reducing termini are possible.

Several different approaches have been proposed to avoid or at least mitigate these problems. For example, alkaline peeling may be prevented by adding $NaBH_4$ to the alkaline solvent, while alkaline extraction may be circumvented by using reagents such as *N*-methylmorpholine *N*-oxide, which dissolves cell walls completely at temperatures above 120 degrees C, allowing fractionation of the dissolved polysaccharides by selective precipitation. However, such processes may lead to polymer degradation.

Alkaline extraction also leads to the undesirable removal of cross-linkable phenolic acid ester groups (e.g., ferulic acid ester groups), with an attendant loss is yield and quality (with respect to gelling potential) of the gelling hemicellulose products.

In WO 93/10158, some of the problems associated with alkaline extraction are addressed by the selection of well defined process conditions. The process permits the solubilization of the polysaccharide whilst retaining most of the ferulic acid substituents on the polysaccharide side branches. The conditions selected for the extraction are similar to those used to saponify lignins and extract proteins from plant tissues. However, the process still co-extracts undesirable phenolic compounds and proteins which must be removed at later stages, and the use of alkali in the procedure necessitates the economically undesirable use of alcohol to precipitate the hemicellulose from the phenolic extract liquor.

In addition, the process can result in the formation of undesirable flavour compounds and protein degradation products that produce bitter off-flavours which persist when the product is incorporated into a foodstuff.

It has now been recognized that alkali-induced solubilisation of gelling hemicellulose is achieved by selective hydrolysis of the more alkali labile acetyl groups located on the main polysaccharide chain. These groups confer insolubility on the hemicellulose macromolecule. However, attempts to completely hydrolyse the acetate ester and so achieve

a higher degree of solubility and yield of product leads to the undesirable removal of ferulic acid side groups from the polymer.

Thus, the existing alkali-based processes balance the removal of acetyl substituents with the preservation of feruloylate esters of the hemicellulose. As such, the yield *or* the quality of the product is compromised as the ferulic acid and acetate content are both linked to extraction conditions. Attempts to increase the yield of soluble polysaccharide lead to the formation of a process artefact (non-gelling hemicellulose) which represents the native hemicellulose that could not be solubilised without complete co-hydrolysis of the cross-linkable phenolic acid substituents together with the acetyl ester groups.

There is therefore a need for improved processes for isolating functional hemicellulosic compositions from hemicellulosic starting materials which do not exhibit these undesirable properties.

It has now been discovered that acetyl esterases can be used to facilitate the extraction of gelling hemicelluloses whilst avoiding the problems associated with alkaline extraction. Treatment with acetyl esterase can selectively remove acetate esters present on the gelling hemicelluloses, so increasing their solubility and permitting the use of mild conditions (e.g., aqueous extraction at or around neutral pH, for example between pH 5 and 9) for the extraction of the gelling hemicelluloses.

The enzymic extraction process significantly improves the yield of gelling hemicelluloses, since for the first time the solubility of the gelling hemicellulose can be controlled (*via* control over the acetyl ester content) independently of the cross-linkable phenolic (e.g., ferulic) acid ester content.

It has also been found that the enzymic extraction processes of the invention also limits the co-extraction of contaminating phenols and proteins to a minimum, whilst avoiding the requirement for an alcohol precipitation stage. Thus, the direct extraction and drying of the hemicellulose can be achieved which reduces process costs considerably. In addition, depending on the process conditions, enzymic treatment can modify the solubility of a hemicellulose composition. As a result, this process enables maximisation of the yield *and* complete control over the final composition of the functional hemicellulose.

It has also been found that the utility of the enzymic extraction processes extend beyond the extraction of gelling hemicelluloses alone, since the removal of hemicelluloses from a wide variety of hemicellulosic starting materials (e.g., plant material) under mild, non-alkaline (or between e.g., pH 5 and 9) conditions yields a unique residue which itself represents a valuable source of novel products and co-products.

Another aspect of the invention relates to the recognition that for some applications the strength of the gels/viscous liquids produced by oxidative gelation must be controlled.

In WO 93/10158, it is proposed that control over viscoelastic properties be achieved by the addition of sugar, salts or alcohols or by treatment with carbohydrase enzymes. This

document goes on to teach that the frequency of ferulate bridges within the polysaccharide network also influences the visoelastic properties and proposes that the extent of ferulate cross linking be controlled at the level of the reaction conditions of the peroxide and oxygenase used to promote oxidative gelation (for example, by limiting the peroxide concentration).

However, the level of control over viscoelastic properties obtainable by such measures is limited, and there remains a need for reliably controlling or pre-determining the gel strength/viscosity over a wide range of values.

It has now been found that the viscoelastic properties of the gels/viscous liquids produced by oxidative gelation of gelling hemicelluloses may be achieved by treating the gelling hemicellulose starting material with ferulic acid esterase.

This enzymic treatment may be carried out under condensing conditions (e.g., conditions of low water activity) to generate ferulic acid hemicellulose esters and so effect an increase in crosslinking potential (and ultimate gel strength). Alternatively, the treatment may be carried out under hydrolytic conditions (e.g., conditions of high water activity) to at least partially de-feruloylate the hemicellulosic starting material and so effect a decrease in crosslinking potential (and a decrease in ultimate gel strength).

In this way, ferulic acid esterase may be used to produce gels and/or viscous media having predetermined viscoelastic properties/viscosities simply by controlling the extent to which the hemicellulose is substituted with ferulic acid ester residues.

It should be noted that WO 96/03440 teaches that ferulic acid esterase activity should be eliminated or avoided during the processing of gelling hemicelluloses, in order to avoid hydrolysis of phenolic-substituted cinnamic acid ester linkages and loss of cross-linking potential. This document therefore teaches away from the use of the hydrolytic activity of ferulic acid esterases in the production of gelling hemicelluloses.

WO 96/03440 goes on to teach that ferulic acid esterase can be used under conditions of low water activity to increase the content of ester residues of the phenolic cinnamic acid ester type, and so improve gelling properties. It also teaches that ferulic acid esterase can be used in this mode to derivitize polymers (e.g., polysaccharides such as pectin, arabinan, galactan, cellulose derivatives, gums, β-glucans and starch) which do not contain phenolic residues in order to attach cinnamic ester type groups (e.g., ferulic acid ester groups) and so render them gellable.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention there is provided an industrial process for adjusting the degree of acetyl ester substitution in a hemicellulose comprising the step of treating the hemicellulose with an acetyl esterase.

As used herein, the term "industrial" is used in contradistinction to the known laboratory scale enzymic digests and syntheses that have been undertaken in the course of

academic and commercial research. The term therefore implies the involvement of large scale apparatus (plant) for producing large (commercial) quantities of products over relatively long periods of time (months or years).

The esterase treatment may modify the solubility of the hemicellulose. For example, the acetyl esterase treatment may be carried out under condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters and/or hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulose. Treatment with the acetyl esterase under condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters effects a decrease in solubility of the hemicellulose, while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulosic starting material effects an increase in solubility of the hemicellulose.

The modification of the solubility of the hemicellulose has great significance for the fractionation of various kinds of plant material, and in particular facilitates the extraction of gelling hemicelluloses therefrom. This follows from the fact that the solubility of the hemicellulose can be increased to the extent that highly efficient (in some circumstances essentially quantitative) extraction into water (or buffered aqueous solutions at or around neutral pH, e.g., between pH 6 and 8) can be achieved under mild conditions without: (a) hydrolysing crosslinkable phenolic substituents that may be present on the hemicellulose; and (b) co-extracting undesirable contaminants. The residue remaining forms a particularly useful source of co-products present in a substantially unhydrolysed state, including proteins, starches, β-glucans, celluloses, lignins, phenolic extracts etc.

The invention also contemplates processes which further comprise the step of adjusting the degree of phenolic ester substitution in the hemicellulose *via* treatment with a ferulic acid esterase (the treatment being either sequential or simultaneous with respect to the acetyl esterase treatment).

This optional enzymic treatment may modify the cross-linking potential of the hemicellulose. For example, the ferulic acid esterase treatment may be carried out under condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters and/or hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulose.

In such embodiments, treatment with ferulic acid esterase under condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters may effect an increase in crosslinking potential (and ultimate gel strength), while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulosic starting material may effect a decrease in crosslinking potential (and a decrease in ultimate gel strength).

In another embodiment, the invention relates to a process (e.g., an industrial process) for fractionating a starting material containing hemicellulose to produce one or more

extracts, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material by steps as defined above.

Preferably, the one or more extracts are selected from extracts comprising (or consisting essentially of) hemicellulose (for example, gelling and/or non-gelling hemicellulose), β-glucan, starch, protein, cellulose, phenolic extracts, lignin, wax, cutin and/or suberin and mixtures of any of the foregoing.

Any or all of these extracts may be produced as co-products in an integrated process.

In another aspect, the invention relates to a process (e.g., an industrial process) for producing a hemicellulose having a predetermined solubility, the process comprising the step of adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a starting material containing hemicellulose by steps as defined above.

In yet a further aspect, the invention relates to a process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) any or all of the following: hemicellulose (for example, gelling and/or non-gelling hemicellulose), a hemicellulose gel, β-glucan, starch, protein, cellulose, a phenolic extract, lignin, wax, cutin and/or suberin or mixtures of any of the foregoing, comprising the step of adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a hemicellulosic starting material by steps as defined herein.

Preferably, the synthetic process of the invention produce two or more of the extracts or compositions as co-products.

In the case where treatment with both acetyl and ferulic acid esterases is carried out, the treatment may be conducted simultaneously or sequentially. When conducted sequentially, the hemicellulose or starting material may be first treated with either the acetyl esterase or the ferulic acid esterase. However, in many circumstances it is desirable to first treat with acetyl esterase to facilitate extraction and then treat the extracted hemicellulose with ferulic acid esterase.

Particularly preferred are processes (e.g., industrial processes) for producing compositions comprising (or consisting essentially of) gelling hemicelluloses, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a hemicellulose contained within a starting material, using steps as defined above.

Here again, the acetyl esterase treatment may effect changes in the solubility of the gelling hemicellulose and the optional ferulic acid esterase treatment effects changes in the gelling characteristics of the gelling hemicellulose.

In this particularly preferred embodiment, the acetyl esterase (and optionally ferulic acid esterase) treatment is carried out under condensing conditions (e.g., low water activity) to form acetyl and/or ferulic acid hemicellulose esters, respectively, and/or hydrolytic

conditions (e.g., high water activity) to at least partially de-acetylate and/or de-feruloylate the hemicellulose, respectively. Here, treatment with the acetyl esterase under condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters effects a decrease in solubility, while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulosic starting material effects an increase in solubility.

Optional treatment with ferulic acid esterase under condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters effects an increase in crosslinking potential (and ultimate gel strength), while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulosic starting material effects a decrease in crosslinking potential (and a decrease in ultimate gel strength).

The particularly preferred process for producing gelling hemicelluloses may advantageously further comprise the steps of: (a) providing an acetylated and feruloylated hemicellulosic starting material; (b) treating the starting material with an acetyl esterase to produce an at least partially de-acetylated feruloylated hemicellulosic material; and (c)-extracting the de-acetylated feruloylated hemicellulosic material.

These optional supplementary steps may also be supplemented with yet further steps involving treating the de-acetylated feruloylated hemicellulosic material of step (c) with a ferulic acid esterase (either under condensing conditions, to further feruloylate the hemicellulosic material, or under hydrolytic conditions, to at least partially de-feruloylate the hemicellulose material), and/or treating the de-acetylated material of step (c) or the ferulic acid esterase treated material of step (d) with an acetyl esterase (either under condensing conditions, to acetylate the hemicellulosic material or under hydrolytic conditions, to at least partially de-acetylate the hemicellulose material).

The invention also contemplates a process (e.g., an industrial process) for producing a gelling hemicellulose comprising the steps of: (a) providing an acetylated and/or feruloylated hemicellulosic starting material; (b) at least partially de-acetylating and de-feruloylating the starting material (e.g., by alkaline hydrolysis or by treatment with a ferulic acid esterase and/or an acetyl esterase); (c) at least partially re-feruloylating the de-acetylated and/or de-feruloylated material of step (b) by treatment with a ferulic acid esterase under condensing conditions (e.g. low water activity) to produce an at least partially re-feruloylated hemicellulosic material; and (d) extracting the re-feruloylated hemicellulosic material.

A yet further process for producing a gelling hemicellulose comprises the steps of: (a) providing a hemicellulosic starting material; (b) treating the starting material with a ferulic acid esterase under hydrolytic conditions, to at least partially de-feruloylate the hemicellulose material.

In another aspect, the invention provides a process (e.g., an industrial process) for producing a composition comprising a non-gelling hemicellulose, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester

substitution) in a hemicellulosic starting material using steps as defined above, wherein the acetyl esterase treatment effects changes in the solubility of the gelling hemicellulose and the optional ferulic acid treatment renders the hemicellulose non-gelling.

This latter process may comprise the steps of: (a) providing an acetylated and feruloylated hemicellulosic starting material, and; (b) treating the starting material with an acetyl esterase and/or a ferulic acid esterase to produce an at least partially de-acetylated and de-feruloylated hemicellulosic material.

In this latter process, the starting material may be treated with an acetyl esterase when the process may further comprises the steps of either:

(c) extracting the de-acetylated feruloylated hemicellulosic material: and then

(d) de-feruloylating the de-acetylated feruloylated hemicellulosic material of step (c), for example by alkaline or enzymic hydrolysis (e.g., by treatment with a ferulic acid esterase),

or

(c') treating the de-acetylated feruloylated hemicellulosic material with a ferulic acid esterase to at least partially de-feruloylate the material; and then

(d') extracting the de-acetylated de-feruloylated hemicellulosic material of step (c').

Also contemplated by the invention is a process for producing a composition comprising (or consisting essentially of) a hemicellulose gel comprising the steps of: (a) providing a gelling hemicellulose according to the process described above, and oxidatively gelling the gelling hemicellulose of step (a) to yield a gel or viscous liquid.

In other aspects, the invention contemplated process (e.g., industrial processes) for producing a composition comprising (or consisting essentially of) any or all of the following co-products: β-glucan, starch, protein, cellulose, phenolic extract, lignin, wax, cutin and/or suberin or mixtures of any of the foregoing, the process comprising the steps of: (a) providing a starting material comprising hemicellulose and any or all of the above listed co-products; (b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined herein, and separating the co-product(s) from the hemicellulose.

The invention also contemplates compositions comprising (or consisting essentially of) any or all of hemicellulose (for example, gelling and/or non-gelling hemicellulose), a hemicellulose gel, β-glucan, starch, protein, cellulose, a phenolic extract, lignin, wax, cutin and/or suberin, mixtures of any of the foregoing,
obtainable by the process of any one of the preceding claims.

Other compositions contemplated by the invention include those comprising (or consisting essentially of) a hemicellulose (for example, a gelling or non-gelling hemicellulose), wherein the hemicellulose has a predetermined degree of acetyl ester substitution, or has been enzymatically acetylated and/or feruloylated *in vitro*, or is at least partially enzymatically re-feruloylated *in vitro* following hydrolytic de-feruloylation *in vitro*.

The invention also contemplates a hemicellulosic composition comprising a predetermined ratio of non-gelling to gelling hemicelluloses, as well as an acetylated gelling hemicellulose.

The invention also contemplated gels (which term also includes viscous solutions, as explained above) comprising any of the gelling hemicelluloses of the invention which have been oxidatively gelled.

The aforementioned gels may be provided in hydrated or dehydrated form. The latter products may be rehydrated to form viscous solutions or gels, and such compositions are also contemplated by the invention.

Hemicelluloses for use in the invention

The hemicellulose for use in the processes of the invention may be any hemicellulose meeting the definition set out earlier. In particular, the hemicellulose may be an arabinoxylan, heteroxylan or pectin. In addition, the hemicellulose for use in the processes of the invention may be a synthetic hemicellulose (i.e., a structural analogue of a naturally-occurring hemicellulose synthesised *in vitro* by any chemical/enzymic synthesis or modification).

Thus, any non-cellulosic, non-starch plant polysaccharides may be used in the process of the invention. Thus, the processes of the invention find application in the processing *inter alia* of pentosans, pectins and gums.

Some hemicelluloses are suitable as substrates for oxidative gelation ("gelling hemicelluloses"): such hemicelluloses often have substituents with phenolic groups which are cross-linkable with certain oxidizing agents. These "gelling" hemicelluloses are particularly preferred for use in the invention.

Arabinoxylans, heteroxylans and pectins may also be used. Of the arabinoxylans, particularly preferred are AXFA, AXF, AXA and AX.

Also suitable for use in the invention are pectins, including the true pectins, simple pectins, complex pectins, mesocomplex pectins and gelling pectins (e.g., those obtainable from representatives of the plant family *Chenopodiaceae*, which include beets (e.g., sugar beet), spinach and mangelwurzels). Particularly preferred is sugar beet pectin (for example in the form of sugar beet pulp). Also useful in the invention are treated pectins (as hereinbefore defined).

Enzymes for use in the invention

The esterases for use in the invention may be of fungal, bacterial, eukaryotic or plant (e.g. cereal) origin. Preferred fungal sources include *Aspergillus* spp. (e.g., *A. awamori*, *A. oryzae* or *A. niger*) and *Trichoderma* spp. (e.g., *T. reesei*). Preferred bacterial sources include *Bacillus stearothermophilus*, *B. subtilis*, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Sulfolobus acidocaldarius* and *Streptomyces* spp. (e.g., *S. olivochromogenes*). Preferred plant sources include cereals, mung beans and orange peel.

The plants useful as sources of the enzymes of the invention may be genetically modified plants which express heterologous DNA encoding the esterases of interest (i.e., the acetyl esterase or ferulic acid esterase).

Enzyme cocktails may be useful in some circumstances. Particularly preferred are cocktails of ferulic acid esterases and acetyl xylan esterases. Other useful enzymes for use in such cocktails include amylases, peroxidases, oxidases, arabinofuranosidases and pectinases.

Conveniently, the ferulic acid esterase and/or acetyl esterase is derived from the same source as the hemicellulosic starting material (e.g., comprises or consists essentially of a protein extract co-product).

The acetyl esterase is preferably an acetyl xylan esterase (e.g., E.C.3.1.1.6).

Enzymic treatment conditions

Appropriate conditions for the enzyme treatment steps can be readily determined by those skilled in the art by routine trial and error. The conditions selected will depend *inter alia* on whether the esterases are used synthetically (when conditions of low water activity must be imposed) or hydrolytically (when higher water activities are indicated).

Parameters such as temperature, time and pH will vary according to the source of the enzyme(s) and the concentration of the reactants. However, as a general consideration hydrolytic acetyl esterase treatment is preferably conducted under conditions that will selectively hydrolyse acetate groups but leave feruloylate ester side chains on hemicellulose intact.

Starting materials for use in the invention

Suitable starting materials containing hemicellulose for use in the processes of the invention (either as starting materials in the fractionation processes or as sources of hemicellulose *per se*) typically include plant material of various kinds and any part or component thereof.

Plant materials useful as a starting material in the invention include the leaves and stalks of woody and nonwoody plants (particularly monocotyledonous plants), and grassy species of the family Gramineae. Particularly preferred are gramineous agricultural residues, i.e., the portions of grain-bearing grassy plants which remain after harvesting the seed. Such residues include straws (e.g., wheat, oat, rice, barley, rye, buckwheat and flax straws), corn stalks, corn cobs and corn husks.

Other suitable starting materials include grasses, such as prairie grasses, gamagrass and foxtail. Other suitable sources include dicotyledonous plants such as woody dicots (e.g., trees and shrubs) as well as leguminous plants.

Another preferred source are fruits, roots and tubers (used herein in the botanical sense). The term "fruit" includes the ripened plant ovary (or group thereof) containing the seeds, together with any adjacent parts that may be fused with it at maturity. The term "fruit" also embraces simple dry fruits (follicles, legumes, capsules, achenes, grains, samaras and nuts (including chestnuts, water chestnuts, horsechestnuts etc.)), simple fleshy fruits (berries, drupes, false berries and pomes), aggregate fruits and multiple fruits. The term "fruit" is also intended to embrace any residual or modified leaf and flower parts which contain or are attached to the fruit (such as a bract). Encompassed within this meaning of fruit are cereal grains and other seeds. Also contemplated for use as starting materials are fruit components, including bran, seed hulls and culms, including malt culms. "Bran" is a component of cereals and is defined as a fraction obtained during the processing of cereal grain seeds and comprises the lignocellulosic seed coat as separate from the flour or meal. Other suitable component parts suitable as starting materials include flours and meals (particularly cereal flours and meals, and including nonwoody seed hulls, such as the bracts of oats and rice).

The term "root" is intended to define the usually underground portion of a plant body that functions as an organ of absorption, aeration and/or food storage or as a means of anchorage or support. It differs from the stem in lacking nodes, buds and leaves. The term "tuber" is defined as a much enlarged portion of subterranian stem (stolon) provided with buds on the sides and tips.

Preferred lignocellulosic starting materials include waste stream components from commercial processing of crop materials such as various beets and pulps thereof (including sugar beet pulp), citrus fruit pulp, wood pulp, fruit rinds, nonwoody seed hulls and cereal bran. Suitable cereal sources include maize, barley, wheat, oats, rice, other sources include pulses (e.g., soya), legumes and fruit.

Other suitable starting materials include pollen, bark, wood shavings, aquatic plants, marine plants (including algae), exudates, cultured tissue, synthetic gums, pectins and mucilages.

Particularly preferred as a starting material is testaceous plant material, for example waste testaceous plant material (preferably containing at least about 20% of arabinoxylan and/or glucoronoarabinoxylan).

The starting material may be treated directly in its field-harvested state or (more usually) subject to some form of pre-processing. Typical pre-processing steps include chopping, grinding, cleaning, washing, screening, sieving etc.

Preferably, the starting material is in a substantially ground form having a particle size of not more than about 100 microns. It may be air classified or sieved (for example to reduce the level of starch). Alternatively, or in addition, the starting material may be treated with enzymes to remove starch (e.g., alpha- and/or beta-amylase). The starting material may also be pre-digested with a carbohydrase enzyme to remove β-glucan.

Suitable washing treatments include washing with hot water or acid (e.g., at a pH of 3-6, e.g., about 5). This at least partially separates protein. Other pre-treatments include protease treatment.

Downstream processing in the extraction of gelling and non-gelling hemicelluloses

As discussed above, the process of the invention may be applied to increase the solubility of both gelling and non-gelling hemicelluloses such as arabinoxylans (including AX and AXF). As a result, extraction of such hemicelluloses is greatly facilitated, and they may be essentially extracted directly into water, non-alkaline (or solutions having a pH of less than 9, e.g., between pH 5 and 9) solutions or an aqueous buffer systems at or around neutral pH.

Once so-extracted, the hemicelluloses may be further processed to concentrate, purify or simply isolate the hemicellulose from the unextracted residue.

Particularly preferred in the preparation of both gelling and non-gelling hemicelluloses (such as AX and AXF) are processes which avoid the use of alcohol precipitation, so avoiding the costs associated with this step.

Preferred processing steps include any of centrifugation, filtration (e.g., ultrafiltration or filtration of vega clay), precipitation (e.g., isoelectric precipitation), chromatography (e.g., silica hydrogel and/or ion exchange chromatography).

Although not preferred, alcohol (e.g., IMS, methanol, ethanol or iso-propanol) precipitation, for example with up to 30% v/v alcohol, may be employed.

Particularly preferred is ultrafiltration or concentration by spray or freeze drying, vacuum rotary drying or ammonium sulphate precipitation.

Any of the aforementioned processes may be applied directly to the aqueous extract of the enzyme modified hemicellulose. Particularly preferred is direct spray or freeze drying from a non-alkaline extract of enzyme-modified material followed by drying, in the absence of an alcohol precipitation step.

Other treatments include desalting treatments, for example dialysis or tangential flow ultrafiltration.

The extracted hemicellulose may be dried as a terminal step, either before or after oxidative gelation (in the case of gelling hemicelluloses). Dried preparations may be supplemented with carriers or dispersants, such as glucose.

Applications

The hemicellulose products (i.e., the gels, dehydrated gels, rehydrated dehydrated gels, non-gelling hemicelluloses, gelling (but ungelled) hemicelluloses and viscous liquids of the invention find a variety of applications various therapeutic, surgical, prophylactic, diagnostic and cosmetic (e.g., skin care) applications.

For example, the aforementioned materials may be formulated as a pharmaceutical or cosmetic preparation or medical device, for example selected from: a wound plug, wound dressing, wound debriding system, controlled release device, an encapsulated medicament or drug, a lotion, cream (e.g., face cream), suppository, pessary, spray, artificial skin, protective membrane, a neutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix. The non-gelling, gelling and gelled hemicelluloses (e.g., AX, AXF and gelled AXF) are particularly useful as agents which maintain the integrity of the gut wall lining, and as agents for coating the luminal wall of the gastrointestinal tract. They may therefore fins particular application in animal feeds and in the treatment of gastrointestinal disorders.

In such embodiments the material, gel or viscous medium of the invention may further comprising an antibiotic, electrolyte, cell, tissue, cell extract, pigment, dye, radioisotope, label, imaging agent, enzyme, co-factor, hormone, cytokine, vaccine, growth factor, protein (e.g., a therapeutic protein), allergen, hapten or antigen (for e.g., sensitivity testing), antibody, oil, analgesic and/or antiinflammatory agent (e.g., NSAID).

Thus, the above-listed materials find application in therapy, surgery, prophylaxis or diagnosis, for example in the treatment of surface (e.g., skin or membrane lesions, e.g., burns, abrasions or ulcers). In a particularly preferred embodiment, the invention contemplates a wound dressing comprising the above listed materials of the invention, for example in the form of a spray. Such wound dressings are particularly useful for the treatment of burns, where their great moisture retaining properties help to prevent the wound drying out.

Particularly preferred for such application is a self-gelling liquid comprising gelling hemicellulose supplemented with glucose and peroxidase and/or oxidase enzymes which gels on contact with oxygen in the air. Such compositions can be provided in the form of oxygen-free liquids in airtight containers which can be sprayed onto the skin, whereupon the liquid gels after exposure to the air. Such composition may advantageously be formulated so as to produce a slight excess of hydrogen peroxide on exposure to oxygen, so that a sterilizing, antibacterial, bacteriostatic and/or cleansing effect is obtained which helps promote healing.

The invention also contemplates water absorbent nappies, diapers, incontinence pads, sanitary towels, tampons and panty liners comprising the above-listed materials, as well as

domestic and industrial cleaning or liquid (e.g., water) recovery operations (e.g., in the oil industry).

Alternatively, the gels of the invention can be provided in the form of hydrated or dehydrated sheets or pellicles for application to various internal or external surfaces of the body, for example during abdominal surgery to prevent adhesions.

Other applications include enzyme immobilizing systems, brewing adjuncts and bread improvers.

The materials listed above also find application as a foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or food dressing. Such products are preferably selected from crumb, alginate replacer, cottage cheeses, aerosol toppings, frozen yoghurts, milk shakes, ice cream, low calorie products such as dressings and jellies, batters, cake mixes, frozen chips, binders, gravies, pastas, noodles, doughs, pizza toppings, sauces, mayonnaise, jam, preserve, pickles, relish, fruit drinks, a clouding agent in drinks, syrups, toppings and confectionary (e.g. soft centres), petfood (wherein the gel e.g., acts as a binder), a flavour delivery agent, a canning gel, fat replacer (e.g., comprising macerated gel), a coating, a glaze, a bait, a binder in meat and meat analogue products (for example vegetarian products), an edible adhesive, a gelatin replacer or dairy product or ingredient (e.g., a yoghurt supplement).

When used as a fat replacer the gel of the invention is preferably macerated to optimize its mouthfeel and fat mimetic properties.

The ungelled gellable hemicelluloses and the non-gelling hemicelluloses find particular utility as biodegradable gums and adhesives, e.g., for use in the paper and packaging industries.

Nongelling hemicelluloses (for example, AX) also find particular application as stabilizers, thickeners and gelatin replacers. They have excellent mouthfeel and texture when used in, for example, mousses and other dairy products.

The ungelled (but gellable) hemicelluloses (e.g., AXF) find particular application as clouding agents (e.g., in drinks), as film forming agents (e.g., in moisture barriers), glazes, edible adhesives and other functional food ingredients.

The cellulose fibre is usually bleached prior to use. It has high water holding capacity, and dispersions may be sheared to produce highly viscous pastes. Particularly preferred applications for this (co)product include dressings (e.g., as a modified starch replacer), yogurts and coatings (and especially batters), where it may act as a crisping agent.

The protein (co)products of the invention have been found to exhibit excellent organoleptic qualities (particularly when digested to varying extents with a protease). Moreover, they have an excellent amino acid profile and are particularly nutritious, being superior to gluten in many respects. Without wishing to be bound by any theory, it is thought that the protein (co)products of the invention derived from starting materials

comprising bran comprise non-storage protein derived from the endosperm of the plant from which the bran was produced.

The protein co-product may be formulated as: (a) an emulsifier; (b) a binder; (c) a whipping agent; (d) a soya analogue; (e) a milk analogue; (f) a protein isolate or concentrate; (g) a flavouring agent; (h) a dehydrated beverage; (i) a roux or roux blanc; (j) a moisture barrier; (k) an alcoholic beverage (e.g., a beer, lager or stout). For some applications, it is preferred that the protein co-product be at least partially digested, conveniently by the protease treatment applied to the starting material (e.g., bran) or hemicellulose extract in the main process stream.

A particularly preferred application for the protein co-product is as a foam stabilizer (or head retention agent) for use in the brewing industry (e.g., in drinks such as lagers, beers and stouts). In these applications, the protein co-product helps stabilize and retain the foamy head traditionally associated with many such drinks. For these applications, the protein is preferably partially digested to yield peptides of between 10 and 50 kD (for example, between 15-30 kD), and protein co-products from wheat-base starting materials (particularly wheat bran starting materials) have been found to perform particularly well.

The various other co-products of the invention (including the β-glucan, starch, protein, cellulose, phenolic extracts, lignin, wax, cutin and/or suberin) find application as foods, food ingredients, food bases, food additives or functional food ingredients. They also find application in various forms of therapy (particularly wound healing).

Particularly preferred in the latter respect are the phenolic extracts of the invention, which also find particular utility as flavouring agents (e.g., vanilla flavourings).

Some of the phenolic extracts and/or waxes, cutins and/or suberins find particular utility as pesticides or crop protection agents.

The invention will now be further illustrated by way of specific Examples, which are purely illustrative and not intended to limit the scope of the invention in any way.

EXAMPLE 1

10 g of maize bran is incubated at pH 5 and 35 degrees Centigrade in 500 ml of water containing 200 units of ferulic acid esterase from *Aspergillus niger* (1 unit = 1 micromole ferulic acid liberrated/min). Free ferulic acid is monitored in the supernatant liquor by absorbance at 310 nm. Ferulic acid is released from the maize bran as below and the properties of isolated AXF gels (2% w/w, gelled using peroxidase/hydrogen peroxide) compared:

Time (min)	Relative amount of free FA	Gel strength
15	15	high
30	50	medium
60	70	low
120	100	nil (liquid)

EXAMPLE 2

100 g of wheat bran is incubated with 500 units of acetylxylan esterase derived from barley malt in 5 litres of water at pH 5 and 50 degrees Centigrade. The extraction of AXF is monitored over 3 hrs by SE-HPLC on the centrifuged supernatant.

Time (min)	Yield of AXF (%)
15	0
45	1
90	3
180	6

All AXF recovered was of high gel strength when gelled (at 2% w/w) using peroxidase/hydrogen peroxide.

CLAIMS:

1. An industrial process for adjusting the degree of acetyl ester substitution in a hemicellulose comprising the step of treating the hemicellulose with an acetyl esterase.

2. A process according to Claim 1 wherein the esterase treatment modifies the solubility of the hemicellulose.

3. A process according to Claim 1 or Claim 2 wherein the acetyl esterase treatment is carried out under:

(a) condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters; and/or

(b) hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulose.

4. A process according to Claim 3 wherein treatment with the acetyl esterase under condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters effects a decrease in solubility of the hemicellulose, while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulosic starting material effects an increase in solubility of the hemicellulose.

5. A process according to any one of the preceding claims further comprising the step of adjusting the degree of phenolic ester substitution in the hemicellulose *via* treatment with a ferulic acid esterase (the treatment being either sequential or simultaneous with respect to the acetyl esterase treatment).

6. A process according to Claim 5 wherein the adjustment modifies the cross-linking potential of the hemicellulose.

7. A process according to Claim 5 or Claim 6 wherein the ferulic acid esterase treatment is carried out under:

(a) condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters; and/or

(b) hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulose.

8. A process according to Claim 7 wherein treatment with ferulic acid esterase under condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters effects an increase in crosslinking potential (and ultimate gel strength), while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulosic starting material effects a decrease in crosslinking potential (and a decrease in ultimate gel strength).

9. A process (e.g., an industrial process) for fractionating a starting material containing hemicellulose to produce one or more extracts, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester

substitution) in the hemicellulose of the starting material by steps as defined in any one of Claims 1 to 8.

10. A process according to Claim 9 wherein the one or more extracts are selected from extracts comprising (or consisting essentially of):

(a) hemicellulose (for example, gelling and/or non-gelling hemicellulose); or
(b) β-glucan; or
(c) starch; or
(d) protein; or
(e) cellulose; or
(f) a phenolic extract; or
(g) lignin; or
(h) wax, cutin and/or suberin; or
(i) mixtures of any of the foregoing.

11. A process (e.g., an industrial process) for producing a hemicellulose having a predetermined solubility, the process comprising the step of adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a starting material containing hemicellulose by steps as defined in any one of Claims 1 to 8.

12. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of):

(a) hemicellulose (for example, gelling and/or non-gelling hemicellulose); or
(b) a hemicellulose gel; or
(c) β-glucan; or
(d) starch; or
(e) protein; or
(f) cellulose; or
(g) a phenolic extract; or
(h) lignin; or
(i) wax, cutin and/or suberin; or
(j) mixtures of any of the foregoing,

comprising the step of adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a hemicellulosic starting material by steps as defined in any one of Claims 1 to 8.

13. A process according to any one of Claims 9, 10 or 12 wherein the process produces two or more of the extracts or compositions as co-products.

14. A process according to any one of the preceding claims wherein the hemicellulose is first treated with:

(a) the acetyl esterase; or
(b) the ferulic acid esterase.

15. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) a gelling hemicellulose, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a hemicellulose contained within a starting material, the process using steps as defined in any one of Claims 1 to 8, wherein for example the acetyl esterase treatment effects changes in the solubility of the gelling hemicellulose and the optional ferulic acid esterase treatment effects changes in the gelling characteristics of the gelling hemicellulose.

16. A process according to Claim 15 wherein the acetyl esterase (and optionally ferulic acid esterase) treatment is carried out under:

(a) condensing conditions (e.g., low water activity) to form acetyl and/or ferulic acid hemicellulose esters, respectively; and/or

(b) hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate and/or de-feruloylate the hemicellulose, respectively.

17. A process according to Claim 16 wherein:

(a) treatment with the acetyl esterase under condensing conditions (e.g., low water activity) to form acetyl hemicellulose esters effects a decrease in solubility, while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-acetylate the hemicellulosic starting material effects an increase in solubility; and

(b) the optional treatment with ferulic acid esterase under condensing conditions (e.g., low water activity) to form ferulic acid hemicellulose esters effects an increase in crosslinking potential (and ultimate gel strength), while treatment under hydrolytic conditions (e.g., high water activity) to at least partially de-feruloylate the hemicellulosic starting material effects a decrease in crosslinking potential (and a decrease in ultimate gel strength).

18. A process according to any one of Claims 15 to 17 further comprising the steps of:

(a) providing an acetylated and feruloylated hemicellulosic starting material;

(b) treating the starting material with an acetyl esterase to produce an at least partially de-acetylated feruloylated hemicellulosic material;

(c) extracting the de-acetylated feruloylated hemicellulosic material (for example by direct extraction into a non-alkaline aqueous solution or a solution having a pH of less than 9, e.g., between 5 and 9), e.g., at a pH of below about 8).

19. A process according to Claim 18 comprising the further steps of:

(d) treating the de-acetylated feruloylated hemicellulosic material of step (c) with a ferulic acid esterase, either:

(i) under condensing conditions, to further feruloylate the hemicellulosic material; or

(ii) under hydrolytic conditions, to at least partially de-feruloylate the hemicellulose material, and/or

(e) treating the de-acetylated material of step (c) of Claim 18 or the ferulic acid esterase treated material of step (d) of Claim 19 with an acetyl esterase, either:

(i) under condensing conditions, to acetylate the hemicellulosic material; or

(ii) under hydrolytic conditions, to at least partially de-acetylate the hemicellulose material.

20. A process (e.g., an industrial process) for producing a gelling hemicellulose comprising the steps of:

(a) providing an acetylated and/or feruloylated hemicellulosic starting material;

(b) at least partially de-acetylating and de-feruloylating the starting material (e.g., by alkaline hydrolysis or by treatment with a ferulic acid esterase and/or an acetyl esterase);

(c) at least partially re-feruloylating the de-acetylated and/or de-feruloylated material of step (b) by treatment with a ferulic acid esterase under condensing conditions (e.g., low water activity) to produce an at least partially re-feruloylated hemicellulosic material;

(d) extracting the re-feruloylated hemicellulosic material.

21. A process for producing a composition comprising (or consisting essentially of) a gelling hemicellulose comprising the steps of:

(a) providing a hemicellulosic starting material;

(b) treating the starting material with a ferulic acid esterase under hydrolytic conditions, to at least partially de-feruloylate the hemicellulose material.

22. A process (e.g., an industrial process) for producing a composition comprising a non-gelling hemicellulose, the process comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in a hemicellulosic starting material using steps as defined in any one of Claims 1 to 8, wherein the acetyl esterase treatment effects changes in the solubility of the gelling hemicellulose and the optional ferulic acid treatment renders the hemicellulose non-gelling.

23. A process according to Claim 21 comprising the steps of:

(a) providing an acetylated and feruloylated hemicellulosic starting material;

(b) treating the starting material with an acetyl esterase and/or a ferulic acid esterase to produce an at least partially de-acetylated and de-feruloylated hemicellulosic material.

24. A process according to Claim 23 wherein the starting material is treated with an acetyl esterase and the process further comprises the steps of either:

(c) extracting the de-acetylated feruloylated hemicellulosic material; and then

(d) de-feruloylating the de-acetylated feruloylated hemicellulosic material of step (c), for example by alkaline or enzymic hydrolysis (e.g., by treatment with a ferulic acid esterase),

or

(c') treating the de-acetylated feruloylated hemicellulosic material with a ferulic acid esterase to at least partially de-feruloylate the material; and then

(d') extracting the de-acetylated de-feruloylated hemicellulosic material of step (c').

25. A process for producing a composition comprising (or consisting essentially of) a hemicellulose gel comprising the steps of:

(a) providing a gelling hemicellulose according to a process as defined in any one of Claims 15 to 21;

(b) oxidatively gelling the gelling hemicellulose of step (a) to yield a gel or viscous liquid.

26. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) a β-glucan, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and β-glucan;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the β-glucan from the hemicellulose.

27. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) starch, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and starch;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the starch from the hemicellulose.

28. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) a protein, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and protein;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the protein from the hemicellulose.

29. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) cellulose, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and cellulose;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the cellulose from the hemicellulose.

30. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) a phenolic extract, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and a phenolic material;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the phenolic material from the hemicellulose to produce a phenolic extract.

31. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) lignin, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and lignin;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the lignin from the hemicellulose.

32. A process (e.g., an industrial process) for producing a composition comprising (or consisting essentially of) wax, cutin and/or suberin, the process comprising the steps of:

(a) providing a starting material comprising hemicellulose and wax, cutin and/or suberin;

(b) adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material using steps as defined in any one of Claims 1 to 8, and

(c) separating the wax, cutin and/or suberin from the hemicellulose.

33. A composition comprising (or consisting essentially of):

(a) hemicellulose (for example, gelling and/or non-gelling hemicellulose); or

(b) a hemicellulose gel; or

(c) β-glucan; or

(d) starch; or

(e) protein; or

(f) cellulose; or

(g) a phenolic extract; or

(h) lignin; or

(i) wax, cutin and/or suberin; or

(j) mixtures of any of the foregoing,

obtainable by the process of any one of the preceding claims.

34. A composition comprising (or consisting essentially of) a hemicellulose (for example, a gelling or non-gelling hemicellulose), wherein the hemicellulose has a predetermined degree of acetyl ester substitution.

35. A composition comprising (or consisting essentially of) a hemicellulose (for example, a gelling or non-gelling hemicellulose), wherein the hemicellulose has been enzymatically acetylated and/or feruloylated *in vitro*.

36. A composition comprising (or consisting essentially of) a gelling hemicellulose, wherein the hemicellulose is at least partially enzymatically re-feruloylated *in vitro* following hydrolytic de-feruloylation *in vitro*.

37. A composition comprising a predetermined ratio of non-gelling to gelling hemicelluloses.

38. An acetylated gelling hemicellulose.

39. A gel comprising the composition of any one of Claims 34-38 which has been oxidatively gelled.

40. The gel of Claim 39 in dehydrated form.

41. A rehydrated gel as defined in Claim 40.

42. A method for increasing the yield of gelling hemicellulose extracted from a hemicellulosic starting material comprising adjusting the degree of acetyl ester substitution (and optionally the degree of phenolic ester substitution) in the hemicellulose of the starting material by steps as defined in any one of Claims 1 to 8.

43. An industrial installation for conducting the process of any one of Claims 1 to 32 or the method of Claim 42.

INTERNATIONAL SEARCH REPORT

In. itional Application No
PCT/US 98/17728

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/14 C08B37/06 C08L5/14 C08B30/04 D21C1/00
C08H1/00 C08H5/00 C08H5/02 C07G1/00 C07K1/00
C07G17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 27221 A (NOVO NORDISK A/S) 31 July 1997 see page 3, line 20 - page 4, line 20 see page 5, line 34 - page 6, line 12 see page 6, line 27 - page 7, line 18 see page 9, line 20 - page 10, line 10 see page 11, line 5 - line 10 ---	1-8, 15-17, 25,34, 35,39,43
X	WO 95 02689 A (NOVO NORDISK A/S) 26 January 1995 see page 3, line 8 - line 13 see page 16, line 1 - line 28 ---	1-4, 9-12,22, 35,43
A	-/--	25-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 1998	18/12/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

In ational Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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